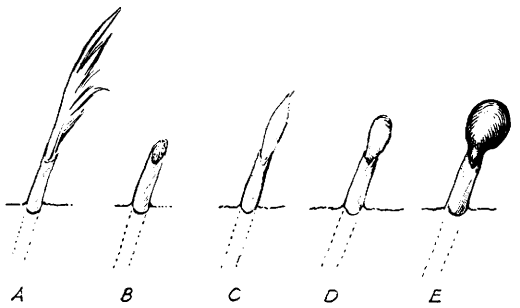


FIG. 2. Diagram of a feather (a) normal; (b) feather has been plucked leaving the shaft; (c) feather regrowing normally; (d) degenerating feather observed after frequent plucking; (e) tumor developing from a feather follicle.

is covered by a narrow layer of squamous epithelial cells (Fig. 1C and D).

Discussion. The skin of ducks treated with methylcholanthrene in acetone shows a wide variety of both histologically malignant and benign neoplasms. All tumors observed so far in more than 100 ducks have spontaneously disappeared except for those removed for histological study. The characteristics of these regressing tumors will be reported subsequently. The occurrence of a fibroma arising from the feather follicle is infrequent when compared with the occurrence of papillomata and hemangiomata in the skin of the duck. Many fibromata do occur in the skin and subcutaneous tissue of ducks treated with methylcholanthrene; however, their pathogenesis is more difficult to determine than the one arising from the feather follicle.

Ducks of similar age with feathers plucked and unplucked treated with acetone and normal ducks with their feathers plucked and unplucked have been observed for 6 months. No tumors have occurred. Degenerative changes, however, have been found in the feather follicles such as described above.

The duck has proven to be a satisfactory experimental host for the production of tumors with methylcholanthrene. The frequent occurrence of papillomata, squamous cell carcinomata and hemangiomata and the spontaneous regression of all neoplasms in the duck present a challenge in carcinogenesis.

Summary. A fibroma is described as arising from the feather follicle of White Pekin ducks following multiple local applications of methylcholanthrene to the skin.

1. Rigdon, R. H., *Arch. Path.*, 1952, v54, 368.

Received April 9, 1953, P.S.E.B.M., 1953, v83.

Surface Fixation as a Practical Method for Diagnosis of Brucellosis in Man and Animals. (20257)

M. RUIZ CASTANEDA.

From the Department of Medical Research, General Hospital, Mexico, D. F. Mexico.

In spite of the considerable work developing and improving methods for the diagnosis of Brucellosis we still lack procedures which by themselves would detect all cases of human and animal diseases. It has been our experience that the more tests that are used the better the chance of obtaining an accurate diagnosis. It is for this reason that we have added to our routine, a new test, the results of which we consider of interest to report. In a previous paper we described a method for the detection of antigen antibody reactions which, so far, has been found applicable only to Brucella and its antibody(1). For practical purposes it was considered desirable to simplify the test, using material of uniform sensitivity and if possible standardized to produce reactions comparable in intensity to the results of tube agglutination tests. Considerable simplification of the test has been achieved by using antigens suspended in sugar cane syrup which may be printed on filter paper and which remain sensitive for several months. This change in the physical condition of the antigen allows for titration of its sensitivity to Brucella antibody in order to

obtain results similar in significance to parallel tube agglutination tests.

In the present note we wish to present additional details concerning the preparation of the antigen, methods of standardization of the test and the results on human and bovine serum. In all cases the tests have been compared with tube agglutination tests and in the case of human patients with blood culture.

Preparation and standardization of the antigen. The method of staining the antigen has been already described(1). By fractional centrifugation, particles of precipitated haematoxylin are removed and then the suspension of stained *Br. abortus* ("S" form) is centrifuged and the sediment resuspended and centrifuged again to remove foreign material. The packed cells are transferred to a mortar and emulsified with freshly prepared sugar cane syrup. This must be prepared with refined sugar and heating to saturation. About 4 volumes of syrup are necessary to emulsify one volume of the sediment. The antigen is standardized to produce reactions of proper intensity when treated with Brucella-antiserum of known agglutinin titre. Since it is known that reactions performed by the tube dilution method are significant only when above certain titre, it has been our aim to produce antigens sensitive to titres above 1:100. For this purpose the antigen and its sugar content are adjusted after testing several dilutions of the stock antigen either with saline alone or with a mixture of saline and syrup. Various preparations are printed on filter paper (Eaton Dikeman No. 609) cut in pieces of 12 by 6 inches. The prints are made by means of a circular rubber stamp or glass rod 3 mm in diameter at a distance of 1 inch from one of the large ends of the paper. The dried spots are treated with the test serum and also with a negative serum control as indicated below. For practical purposes it is advisable to select the antigen which produces from 50 to 75% fixation with a serum of 1:160 titre. The selected sample is used as a guide in the preparation of the antigen. This is printed on the paper as previously indicated and is ready for use the next day. The printed material should be kept in a dry place at room temperature.

Test. For a single test, paper containing 3 spots is cut transversally from the leaf. Over the middle spot a droplet of unknown serum is placed with a wire loop 3 mm diameter. On each side a positive and negative control are placed with similar loops. Before the serum dries, the paper is hung with its end in a layer of isotonic NaCl solution taking care that the spots remain about half an inch over the surface of the fluid. From 20 to 25 minutes may be required for the absorbed fluid to reach the upper part of the paper. *Readings.* The negative control will show a trail of antigen starting from the spot, to nearly the upper part of the paper (about 4 inches). The positive control will remain fixed. The titre of the unknown serum may be estimated according to its behaviour in relation to the controls. Accordingly, the intensity of the reaction, when positive, may be graded either by the customary 1 to 4 plus or by approximate percentages of fixation.

Results. Table I shows the results of 906 tests performed with serum obtained from 590 patients suffering from brucellosis in whom the diagnosis was proven by the isolation of Brucella from the blood or by an agglutination test of high titre. The cases are classified according to the results of the agglutinin titre as obtained by the tube dilution method. The results of the paper test are classified according to percentages of fixation. As controls we include 221 tests performed on patients suffering from various acute and chronic infections not including brucellosis. About 10% of these cases had agglutinin titres for Brucella ranging from 1:20 to 1:80. There is another group of 100 tests performed with normal human, guinea-pig and rabbit sera.

From this table several observations may be made: 1) No negative "surface fixation" tests were shown in proven cases of active brucellosis. 2) Low titres of agglutinin are usually concomitant with weak "surface fixation" tests. 3) The intensity of the "surface fixation" test increases with increasing agglutinin titres.

Considerable discrepancies, however, have been observed with sera of low agglutinin titres. Nearly 3% of all tests showed a negative agglutination test. The diagnosis, how-

TABLE I. "Surface Fixation" Compared with Tube Agglutination in Human Brucellosis Proven by Blood Culture or High Agglutinin Titres.

Tube agglut. titre	No. of cases	Results of surface fixation: 25% No.	25% %	50% No.	50% %	75% No.	75% %	100% No.	100% %
Negative*	27	16	59.2	7	25.9	4	14.8	0	0
1:20 *	15	12	80.0	1	6.6	0	0	2	13.3
1:40 *	21	13	62.0	5	23.8	2	9.5	1	4.7
1:80 *	77	30	38.9	25	32.5	17	22.1	5	6.5
1:160	113	10	8.8	41	36.3	40	35.4	22	19.5
1:320	194	10	5.0	51	26.6	93	47.9	40	20.5
1:640 and over	459	6	1.3	35	7.7	181	39.4	237	51.6
Total	906 cases of brucellosis.								

Control groups: 1*—221 patients suffering from various infections not including brucellosis, some with agglutinin titres of 1:20 to 1:80. Negative "surface fixation"
2*—100 normal human, guinea pig and rabbit sera. Negative "surface fixation"

* These cases had simultaneous or recent positive blood cultures.

ever, was based on simultaneous or recent isolation of Brucella from the blood as it was the case with the following 3 groups of patients giving titres from 1:20 to 1:80. Since titres below 1:100 have no diagnostic value by themselves, the percentages of cases of active brucellosis which may not be detected by a single tube agglutination test increases to 12%. Since in all these cases antibody may be detected by "surface fixation", such failure to be shown by tube agglutination may depend on the possible interference by blocking phenomena(2). Otherwise, the observation of patients for long periods after the acute phase of brucellosis has shown in general a parallel decrease in intensity of both agglutinin titre and "surface fixation" reaction.

TABLE II. Sample of Survey with "Surface Fixation" and Tube Agglutination on Bovines from the Same Herd.

Serial No.	Agglutinin titre	% of fixation
75	20	0
76	640	100
77	160	50
78	10	0
79	40	0
80	10	0
97	1280	100
98	320	75
99	40	0
100	80	50
101	20	25
102	80	50

TABLE III. "Surface Fixation" and Agglutination Test with Sera from 716 Bovines from Several Herds.

No. of animals tested	Agglutinin titre	Results according to % of fixation: 0	25	50	75	100
318	Negative	318	—	—	—	—
136	1:10	134	2	—	—	—
95	1:20	86	8	1	—	—
61	1:40	45	13	3	—	—
25	1:80	11	8	5	1	—
81	>100	2	8	15	16	40
716		596	39	24	17	40

A comparative study of "surface fixation" and tube agglutination test performed with bovine serum has given similar results, although discrepancies cannot be discussed on the same basis as in human patients because of the impossibility to establish the diagnosis in random cases by means of blood culture. About 1,500 comparative tests have been performed and the results summarized in Tables II and III.

Because of the way in which the paper test has been standardized one would not expect clear cut positive reactions with sera of titres below 1:100. However, in Table II it may be seen that in some cases fixation as high as 50% may be shown by sera of titres of 1:80. The interpretation of such results is difficult because of reasons previously given.

It is of interest to observe Table III in which a group of 716 bovines were tested by the 2 methods. The correlation between negative "surface fixation" and negative tube agglutination test was correct, but nearly 13% of the sera with agglutination tests from 1:10

to 1:80 produced positive "surface fixation" tests of different intensity. These discrepancies may be due to inhibitory factors in the agglutination test which have no influence on the paper test.

It may be noticed that stronger "surface fixation" tests are to be found in sera of titres above 1:100. On the other hand, 2 out of 81 cases with significant tube agglutination tests failed to produce "surface fixation" reaction. The only possible explanation of the latter discrepancy may be related to the rather low sensitivity of the paper to sera with agglutinin titres close to 1:100.

Summary. Attempts have been made to standardize the "surface fixation" test for the diagnosis of brucellosis and at the same time make it a practical rapid and dependable procedure. So far, it has been found to possess remarkable specificity in human brucellosis as judged by comparison with blood cultures. This test may be applicable to the detection of bovine reactors.

1. Castaneda, M. Ruiz, PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 46.
2. Griffitts, J. J., *Pub. Health Rep.*, 1947, v62, 865.

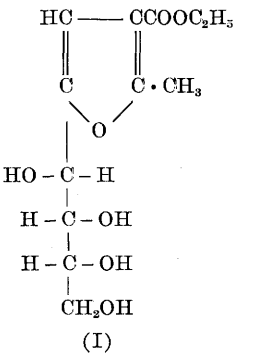
Received April 13, 1953. P.S.E.B.M., 1953, v83.

Biosynthesis of Vitamin C: A New Precursor. (20258)

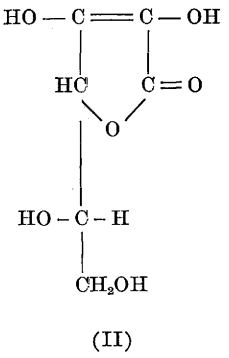
M. C. NATH, B. BELAVADY, V. K. SAHU, AND R. P. CHITALE.
From the University Department of Biochemistry, Nagpur, India.

Much work has been done on the biosynthesis of vit. C, but the precise mechanism is still to be revealed. Ray(1), Smythe, and King(2) and several others have indicated that hexose sugars and sugar acids are in some way concerned in the biosynthesis, and according to Mentzer and Urbain(3) lipids also can serve as the precursor of ascorbic acid in the body of the rat.

Acetoacetate, an intermediary metabolite of lipids found by Nath and associates(4,5) to cause an immediate rise in the blood glucose and decrease in glucose tolerance, has recently been reported(6) to cause a sudden increase in vit. C of blood of rat. Because glucose as well as acetoacetate, in small doses, accelerated biosynthesis of vit. C, it was thought desirable to see the combined effect of the two in germinating mung bean (*Phaseolus mungo*), as well as in the body of the rat. It has recently been briefly reported(7) that though acetoacetate alone has a retarding effect on biosynthesis of vit. C in the germinating mung bean, acetoacetate and glucose added to the medium in equimolecular proportion cause a gradual rise of vit. C. The condensation product of the two(8) which Gonzales(9) showed to be 2-tetrahydroxy butyl 5-methyl 4-carbethoxy furan (I) sharply raised the vit. C (II) level of the germinating mung bean from the start of the experiment.



(I)



(II)

Experimental. (A) *Biosynthesis in germinating mung bean.* Glucose and acetoacetate (Na salt) separately or together and the condensation product of the two were added to the medium for germination of mung bean. Seeds were first soaked in glass-distilled water for about 3 hours, then immersed in aqueous media containing these different substances, at pH 7.4. For each experiment 500 seeds of almost equal weight were selected. Potassium meta bi-sulphite (300 P.P.m.) was added to all media as recommended by Kendrick and Downer(10). One hundred seeds were taken when required, the testa removed and ex-